Acta Biomaterialia xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Setd7 and its contribution to Boron-induced bone regeneration in B-MBG scaffolds

Chengcheng Yin [a], Xiaoshi Jia [a], Richard J. Miron [b], Qiaoyun Long [c], Hudi Xu [a], Yan Wei [a], Min Wu [c], Yufeng Zhang [a,*], Zubing Li [a,*]

[a] The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, Wuhan University, Wuhan, China
[b] Department of Periodontology, Cell Therapy Institute, College of Dental Medicine, Nova Southeastern University, Fort Lauderdale, Florida, USA
[c] Hubei Key Laboratory of Cell Homeostasis, Hubei Key Laboratory of Developmentally Originated Disease, Department of Biochemistry and Molecular Biology, College of Life Sciences, Wuhan University, Wuhan, Hubei 430072, China

ARTICLE INFO

Article history:
Received 29 November 2017
Received in revised form 4 April 2018
Accepted 18 April 2018
Available online xxxx

Keywords:
Bone regeneration
Osteoporosis
B-MBG
Setd7
H3K4me3

ABSTRACT

Boron (B), a trace element found in the human body, plays an important role for health of bone by promoting the proliferation and differentiation of osteoblasts. Our research group previously fabricated B-mesoporous bioactive glass (MBG) scaffolds, which successfully promoted osteogenic differentiation of osteoblasts when compared to pure MBG scaffolds without boron. However, the mechanisms of the positive effect of B-MBG scaffolds on osteogenesis remain unknown. Therefore, we performed *in-vivo* experiments in OVX rat models with pure MBG scaffolds and compared them to B-MBG scaffold. As a result, we found that B-MBG scaffold induced more new bone regeneration compared to pure MBG scaffold and examined genes related to bone regeneration induced by B-MBG scaffold through RNA-seq to obtain target genes and epigenetic mechanisms. The results demonstrated an increased expression and affiliation of Setd7 in the B-MBG group when compared to the MBG group. Immunofluorescent staining from our *in vivo* samples further demonstrated a higher localization of Setd7 and H3K4me3 in Runx2-positive cells in defects treated with B-MBG scaffolds. KEGG results suggested that specifically Wnt/β-catenin signaling pathway was highly activated in new bone area associated with B-MBG scaffolds. Thereafter, *in vitro* studies with human bone marrow stem cells (hBMSCs) stimulated by extracted liquid of B-MBG scaffolds was associated with significantly elevated levels of Setd7, as well as H3K4me3 when compared to MBG scaffolds alone. To verify the role of Setd7 in new bone formation in the presence of Boron, Setd7 was knocked down in hBMSCs with stimulation of the extracted liquids of B-MBG or MBG scaffolds. The result showed that osteoblast differentiation of hBMSCs was inhibited when Setd7 was knocked down, which could not be rescued by the extracted liquids of B-MBG scaffolds confirming its role in osteoblast differentiation and bone regeneration. As a histone methylase, Setd7 may be expected to be a potential epigenetic target for new treatment schemes of osteoporosis.

Statement of Significance

Boron-containing MBG scaffold has already been proved to promote bone regeneration in femoral defects of OVX rats by our research group, however, the epigenetic mechanism of Boron's positive effects on bone generation remains ill-informed. In our present study, we found an increased expression and affiliation of Setd7 and H3K4me3 in Runx2-positive osteoblasts in vivo. And *in vitro*, the higher expression of Setd7 enhanced osteogenic differentiation of human BMSCs stimulated by extracted liquids of B-MBG scaffold compared to MBG scaffold, which was associated with the activation of Wnt/β-catenin signaling pathway. Above all, it suggests that Setd7 plays an positive role in osteogenic differentiation and it may become a potential epigenetic target for new schemes for osteoporosis.

* Corresponding authors.
E-mail addresses: zyf@whu.edu.cn (Y. Zhang), lizubing@whu.edu.cn (Z. Li).

https://doi.org/10.1016/j.actbio.2018.04.033
1742-7061/

1. Introduction

Bone defect is a common scenario in clinical practice that originate from tumors, trauma, infections, and genetic malformations. Its treatment resolution remains a prominent challenge in modern medicine, in particular for large defects or defects in patients with systemic diseases such as osteoporosis [1]. In osteoporosis, significant bone loss develops during the aging process due to an imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption [2]. In such a scenario, more difficulty is needed to restore the bone defect as the ability for osteoblasts to promote new bone formation is decreased with respect to the hyper activity of osteoclasts. To resolve these issues, bioactive porous scaffolds have been widely studied to regenerate lost bone [3]. Mesoporous bioactive glass (MBG), as a bioactive scaffold, has been highly researched in recent years showing great potential for bone tissue engineering and drug-delivery applications [4–7]. One such element that can be incorporated into MBG is Boron (B), an essential element found in the human body playing an important role in bone health. Furthermore, Boron has been suggested to mimic the effects of estrogen via stimulating its production in post-menopausal women [8], which is one of the most effective methods to prevent post-menopausal bone loss [9]. Studies have reported that Boron induces early matrix mineralization via calcium deposition and elevation of alkaline phosphatase activity in differentiated rat bone marrow mesenchymal stem cells [10,11]. In our group's previous research [6], it was found that B-MBG scaffolds were an ideal delivery vehicle for dexamethasone (DEX) to promote osteogenic differentiation of osteoblasts. Interestingly, we found that B-MBG scaffolds without DEX could also enhance the proliferation and the gene expression of Col I and Runx2 of osteoblasts, when compared to pure MBG scaffolds. Furthermore, the incorporation of B into MBG scaffolds did not significantly alter the release of Ca^{2+} and SiO_4^{4-} when compared to pure MBG scaffolds, other than the increasing release of BO_3^{3-} in B-MBG scaffold.

Histone methylation by lysine methyltransferase enzymes regulate the expression of genes implicated in lineage specificity and cellular differentiation [12] which is one of the most widely utilized post-transcriptional modifications. It is thought to be an important epigenetic event that is closely associated with cell fate determination and differentiation. SET7/9, as a kind of protein lysine methyltransferases, can methylate both lysine 4 of histone 3 (H3K4) and lysine (s) of more than 30 non-histone proteins, including transcriptional factors, tumor suppressors, and membrane-associated receptors [13]. One study found that during human tooth development, SET7 was expressed in odontoblast layers and dental papilla tissues from the early bell stage. As the mesenchymal stem cells in dental papilla have more potential ability to differentiation than the cells in enamel organ do [14], these results indicated that the expression of setd7-trimethylated H3K4 was associated with transcriptionally active genes. It has been proposed that H3K4me3 might poise developmental regulators for activation upon differentiation [15]. In this scenario, H3K4me3 might make the induction of developmental genes more efficient or more synchronous [16]. It also protects genes from permanent silencing, such as by repelling transcriptional repressors or blocking DNA methylation [17]. Recently, Yang et al. demonstrated that Plant homeodomain finger protein 20 (PHF20), a methyl lysine effector protein could increase the enrichment of H3K4me3 on the promoter of Runx2 followed by increased Runx2 promoter activity, enhanced ALP activity and mineralized nodule formation and expression of osteogenic markers. It was found that PHF20 positively regulates osteoblast differentiation via increasing the expression and activation of Runx2 with enrichment of H3K4me3 [18].

Previously, our group has prepared Sr-MBG scaffolds, which induced higher levels of new bone formation and osteoblast differentiation when compared to MBG scaffolds. It was later found that this was related to the up-regulation of Setd2 expression through activation of the MAPK pathway. In addition, Setd2 regulated ERK activation in a positive feedback system to promote osteoblastic differentiation [19,20]. Similarly, Boron-containing MBG scaffold have been investigated for their ability to promote bone regeneration [21], however, their detailed molecular mechanism of action and associated transcription factors remains largely unknown.

In this study, by means of RNA-seq technology, we found that B-MBG increased protein lysine methyltransferase, Setd7 in the regenerated bone tissue *in vivo* when compared to MBG. Thus, the purpose of this study was to investigate the role of Setd7 during osteoblast differentiation and further investigate the correlation between Setd7, H3K4me3 and new bone formation stimulated by B-MBG.

2. Materials and methods

2.1. Materials and the ovariectomy (OVX) rat model

Preparation of porous Mesoporous bioactive glass (MBG) and Boron-containing MBG (B-MBG) scaffolds and establishment of the osteoporotic animal models had been performed and examined in our previous study [6]. Mature female Wistar rats (8 weeks old, body weight 180–200 g) were purchased and used for this study. All experimental procedures have been approved by the Ethics Committee for Animal Use of the Institute of Biomedical Sciences, under protocol number 134/2012. Firstly, the rats were subjected to bilateral ovariectomy under general anaesthesia by intraperitoneal injection of sodium pentobarbital (2% in weight, 2 mL per kg body weight). After a period of 3 months, according to the previous methods [22], the critical femoral bone defect of 3 mm was made in the OVX rats, then, the bone defects were gently filled with MBG or B-MBG scaffolds. 8 weeks later, the rats were sacrificed by excessive sodium pentobarbital performed by 2 trained staff members following a protocol approved by the Institutional Animal Care and Use Committee (IACUC) and collected the femoral bones.

2.2. Immunohistochemical and immunofluorescent staining

After being fixed with 4% formaldehyde for 48 h, the femoral samples were immersed in 10% EDTA changed three times per week for 12 weeks for decalcification, then gradient dehydrated for embedding in paraffin. To compare the bone regeneration difference of the two scaffolds, Haematoxylin-eosin (H&E) staining, Masson staining and immunohistochemistry staining were performed following the manufacturer's protocols (MXB biotechnologies, China). The primary antibody used for immunohistochemistry staining are as follows: Runx2 (1:800; Abcam ab76956, Cambridge, UK), Setd7 (1:800; ABclonal A9985, U.S.A.), H3K4me3 (1:400, ABclonal A2357, U.S.A.). The sections were visualized by 3,3-diaminobenzidine tetrahydrochloride (DAB) (Zhongshan Biotechnology, Ltd, China). For immunofluorescent staining, the sections were incubated with two primary antibodies of Runx2 and Setd7 or H3K4me3. The anti-mouse secondary antibody with green fluorescent marker and anti-rabbit secondary antibody with red fluorescent marker were bought from Abbkine (U.S.A). The nucleus of cells in tissue were stained using DAPI dye (Zhongshan Biotechnology, Ltd, China) in the mounting medium. The images of all stained sections were captured with

Please cite this article in press as: C. Yin et al., Setd7 and its contribution to Boron-induced bone regeneration in B-MBG scaffolds, Acta Biomater. (2018), https://doi.org/10.1016/j.actbio.2018.04.033